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To determine the number of different receptors expressed by an individual olfactory neuron, and to match a particular receptor gene with a set of specific ligands.

(see attached report)



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PRINCIPAL INVESTIGATOR: Dr. Stuart Firestein

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GRANT TITLE: Single Cell PCR in Olfactory Neurons

AWARD PERIOD: July 1, 1994 through June 30, 1997

OBJECTIVE: To determine the number of different receptors expressed by an individual olfactory neuron, and to match a particular receptor gene with a set of specific ligands.

APPROACH: First isolated olfactory neurons from the tiger Salamander are subjected to whole cell patch clamp to record the responses to panel of odors. This is followed by recovery of the intracellular contents, which are then amplified by RT-PCR using primers designed to detect odor receptor transcripts. The primers are sufficiently degenerate to recognize a large number of potential odor receptor mRNA transcripts.

ACCOMPLISHMENTS: The combination of whole cell patch clamp and PCR on single cells makes use of two frontier technologies. Although patch clamp recordings in single olfactory neurons have become somewhat commonplace, the particular levels of stability and care required in these experiments were at the limit of the existing technology. In addition to finding neurons, which were responsive to the particular odors in our panel, it was critical to preserve the condition of the cell so that mRNA could be reliably recovered. Further precautions were necessary to guard against possible contamination by nuclear DNA. Because the odor receptor genes, like most other GPCRs, are encoded genomically as a single exon there is a constant danger that the results will be tainted by unwanted genomic DNA. Therefore the dissociated cells had to be treated very carefully so as not to disrupt them, the solutions had to be maintained at a high level of purity and, most critical of all, the integrity of the nucleus in cells from which we recorded had to be maintained.

In spite of these difficulties we were able to regularly record odor responses from neurons and in several cases retrieved the intracellular contents. In these cases the mRNA was reverse transcribed with ologo-dT primers and then amplified by PCR. The amplified cDNAs were then subjected to a second PCR with degenerate primers matching conserved amino acid sequence motifs in mammalian Ors. The OR cDNA products of this second PCR reaction were then isolated and sequenced. In five cells we recovered odor receptor transcripts that were of a single odor receptor in each cell, consistent with the notion that a cell expresses only a single odor receptor gene.

However, a serious obstacle to the reliability of these results was the low level of success in the single cell PCR. We were able to recover a cDNA from only about 5% of the cells subjected to the total procedure (n-11 of 200 cells). While we suspect that this was due to the fallibility of the technique it remains possible that the reason so few transcripts were recovered is that the degenerate primers failed to recognize a substantial enough portion of the whole family. This is problematic because it remains a possibility then that in those cells in which a single transcript was recovered additional r4eceptors might have been missed due to the primers. We calculate (by Poisson statistics) that a success rate approaching 85% would be needed to consider the findings in a single cell reliable. Unfortunately, this level is unattainable with current technology. We were further hampered by the lack of a developed molecular biology in the salamander, so that many of the standard controls that would have provided an independent estimate of the PCR reliability were unavailable.

Reluctantly, we conclude that it is impossible to determine the number of receptors expressed by single salamander olfactory neurons. We have however contributed the 12 novel sequences of odor receptors that we cloned in the salamander to the public database.